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Research Report

Effects of soy isoflavone genistein on orthodontic tooth movement in guinea pigs

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ABSTRACT

Background: *Osteoblast and osteoclast are the important factor in periodontal tissue remodeling for the orthodontic treatment success. Resorption process takes place in compression area by osteoclast and apposition in the tension area by osteoblast. In general hormone condition and age affect remodeling process. Estrogen has a high contribution in remodelling process and decreased in elderly individual such as menopausal women. Soybean contains isoflavone genistein which has similar structure and activity to estrogen. Many researchers indicate that isoflavone genistein not only has an inhibitor effect in osteoporosis but also has estrogenic and antiestrogenic effect as well.* ***Purpose:*** *The study aimed to investigate the effect of soybean isoflavone genistein administration on osteoblast and osteoclast cells number in orthodontic tooth movement of young and old guinea pigs.* ***Method:*** *The research was quasi-experimental study with post test only with control design. The experimental animals were 24 male guinea pigs that divided into: young guinea pigs (±4 months old) and old guinea pigs (±2.5 years old). Each group was divided into 4 subgroups for receiving the treatment namely; control, orthodontic treatment, genistein treatment and orthodontic+genistein treatment. All of the subjects were sacrificed at day 7 and the specimens were histologically analyzed using tartrate resistance acid phosphatase (TRAP) and hematoxylin eosin (HE) staining and observed using microscope that connected to obtilab and an image raster program.* ***Result:*** *U Mann-Whitney statistical analysis showed there were significant differences in osteoblast cell numbers; between orthodontic treatment and orthodontic+genistein treatment in the old guinea pigs (p=0.004); between orthodontic treatment in the young guinea pig and orthodontic+genistein treatment in the old guinea pig (p=0.016); between orthodontics treatment and orthodontic+genistein treatment in the young guinea pigs (p=0.025). U Mann-Whitney statistical analysis showed there were significant differences in osteoclast cell numbers: between the orthodontic treatment in the old guinea pig and orthodontics+genistein treatment in the young guinea pigs (p=0.007); between orthodontic treatment group in the young guinea pigs and orthodontics+genistein treatment in the old guinea pigs; between orthodontic treatment and orthodontic+ genistein treatment in the young guinea pigs (p=0.007). All groups administered by genistein the numbers of osteoblast in the surrounding of the tension sites increased, while in the surrounding of the compression sites had less osteoclasts; even, there were no osteoclasts found in some samples.* ***Conclusion:*** *Soybean isoflavone genistein administration on orthodontic tooth movement increased osteoblast numbers in the tension sides and decreased osteoclast numbers in the compression sides.*

Keywords : *isoflavone genistein of soybean; guinea pig; orthodontic tooth movement*

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INTRODUCTION

Orthodontic treatment aims to improve the functionality and aesthetics of teeth, either in children, in adults, or in elderly (seniors). One of the main problems that encourage the orthodontic treatment usually is the presence of malocclusion, especially related with the function, aesthetics, and physiological function of teeth . This means that the orthodontic treatment is performed not only for the aesthetic needs of patients, but also for some functional

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reasons.[1,2] One way of handling the orthodontic treatment of malocclusion is by moving the teeth and jaws into right position.[3]

Based on data obtained from the Ministry of Health in 2004, 60% of Indonesia's population suffers from oral and dental diseases, and malocclusion disorder ranked the second position after dental caries.[4] The prevalence of malocclusion in adolescents in Indonesia is quite high, and the number is relatively still stable, about 80-90%.[5] In 2014, the percentage of malocclusion patients in Indonesia amounted to 80% of the total population.[6] Malocclusion can also be considered as a oral and dental abnormality associated with occlusion. Occlusion can be defined as a contact between under teeth and upper teeth when mouth is closed.[7]

The success of orthodontic treatment depends on the process of periodontium tissue remodeling during the treatment. Periodontium tissue surrounding and supporting the teeth, which main components are osteoblasts, osteoclasts, sementoblas, and a series of collagen fibers with a basis of proteoglycans and glycoprotein.[8] Bone remodeling process that involves apposition and resorption processes is closely associated with the number of osteoclasts and osteoblasts. Bone is a dynamic tissue constantly experiencing apposition and resorption. The peak of the human ossification occurs around the age of 35 years, and will decrease slowly.[9] Mundy also states that the bone mass decreases progressively with age in both men and women starting at the age of 30 years.[10]

Bone formation or apposition in children who are developing is greater than bone resorption, whereas in healthy adults bone apposition and bone resorption are balanced. Menopause and aging process both in men and in women will trigger a decrease in bone formation or apposition compared to bone resorption.[11] The imbalance between bone formation by osteoblasts and bone resorption by osteoclasts in menopausal women is due to hormonal changes, leading to the high rate of osteoporosis-related fractures.[12] Osteoblasts and osteoclasts are two important cells involved in orthodontic tooth movement, so a lot of researches have focused on these cells. Osteoblasts involved in bone formation will appear 40-48 hours after the provision of orthodontic power.[13] A study was done by Boulpaep and Boron cited by Kini and Nandeesh states that after several hours under the pressure, osteoclasts on the side of the periodontal membrane will multiply and begin to resorb bone surface, whereas in areas exposed to traction, osteoblasts will multiply and begin to form new bone layers on the bone walls.[14] Based on the circumstances, therefore, the success of orthodontic treatment is determined by the success of the remodeling process that depends on the quantity and quality of osteoclasts and osteoblasts.[15]

Some phytochemical compounds also known as phytonutrients including phytoestrogens are plant compounds that exist in the daily diet. Phytoestrogens can positively regulate a number of physiological functions in mammalian systems involved in chronic diseases, such as osteoporosis. However, their effects on tooth movement still have not been known certainly.[13]

Natural products containing phytoestrogens are soy isoflavone genistein that can stimulate osteoblast differentiation and new bone formation in osteoporosis treatment. Phytoestrogens are natural estrogenic compounds found in many plants and seeds. This compound has a structure similar to mammalian estrogen.[16] This compound also has estrogenic or antiestrogenic effects.[16,17]

Soy isoflavone genistein is bioactive compounds that have estrogen-like activity. According to a lot of researches, soy isoflavone genistein also plays a role in the prevention and treatment of osteoporosis associated with a decrease in estrogen levels.[12,17,19] Isoflavones, thus, may inhibit bone osteoporosis in women after the menopause.[18] Some derivatives of the soybean have been identified to have a positive effect on bone without triggering side effects.[19] For those reasons, this research aimed to analyze the effects of soy isoflavone genistein on remodeling of tissues supporting teeth in old and young guinea pigs which their teeth were moved orthodontically.

MATERIALS AND METHODS

This research was a quasi-experimental study with posttest control group design. The subjects included 24 male guinea pigs, consisted of 12 young males (age ± 4 months) with an average weight of ± 300 g and 12 old males (age ± 2.5 years) with an average weight of ± 800 grams obtained from LPPT Unit 4 Universitas Gadjah Mada. Guinea pigs were adapted for 5 days by giving a standard diet in the form of pellets and green vegetables as a source of vitamin C. They were kept in a cage at room temperature.

Those young and old samples were randomly divided into four (groups), namely: 1) control group; 2) treatment group given orthodontic treatment; 3) treatment group given isoflavone genistein (genistein treatment); 4) treatment group given both orthodontic treatment and isoflavone genistein treatment. Each tooth moved was lower incisor with an open coil spring with a strength of 35 cN (Figure 1).

Genistein was derived from Genistein Tempe formula (produced by Prof. Mien Karmini from IPB) composed of soybean, tape, flour, sugar, creamer, salt, and baking powder. Genistein orally given to guinea pigs was at doses of 1.2 mg to 0.6 mg daily. On the 7th day, they were sacrificed, and then their mandible was cut. Histological tests on osteoclasts for all groups using tartrate resistance acid phosphatase (TRAP) staining similar to the method of a research conducted by Kalajzic *et al.*[20] and histological tests for all groups on osteoblasts using hematoxylin eosin staining (HE) corresponding to the method of a research conducted by MirHashemi *et al.*[21]

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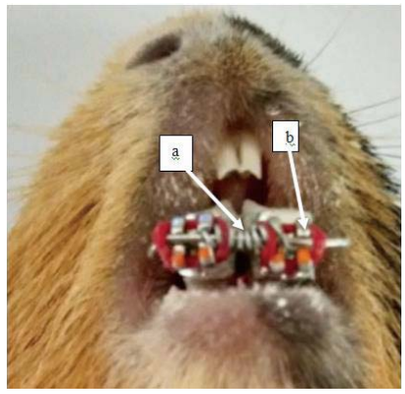

Figure 1. Orthodontic appliance installation of an open coil spring mounted with round wire between two brackets on the incisors of the guinea pigs. Tension site was next to a mesial area of incisors, both right and left. Pressure site was next to a a distal area of incisors, both right and left. a) open coil springs; b) bracket.

Osteoclasts and osteoblasts were observed by a light microscope (Olympus CX21FSI, Tokyo, Japan)) with attachement of obtilab advance. A raster program software was used to calculate them.

RESULTS

Based on the results of both histological observation using optilab, as well as osteoclast and osteoblast calculations in both pressure and tension sites using raster image program to 5 visual fields on each subject research, means and standard deviations of osteoclasts can be seen in Table 1, while means and standard deviations of osteoblasts can be seen in Table 3. Osteoclasts appeared with many red nucleated cells after using TRAP staining, while osteoblasts attached to bone after using HE staining (Figure 2, Figure 3, Figure 4 and Figure 5)

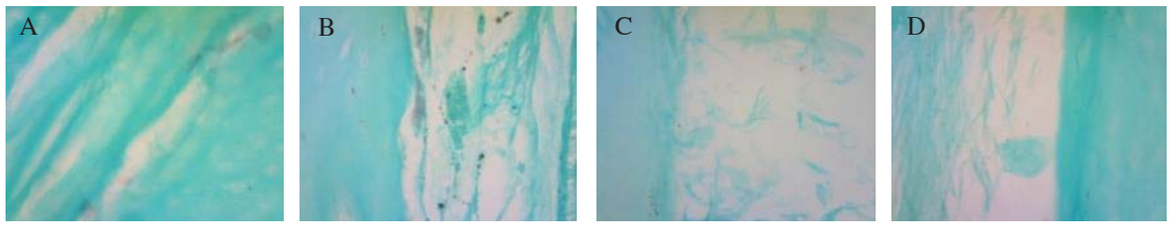

Figure 2. The results of histology test on periodontal tissues of the old guinea pigs, on the 7th day, at 400x magnification, with TRAP staining. A) the control group; B) the orthodontic treatment group; C) the genistein treatment group; D) the orthodontic + genistein treatment group.

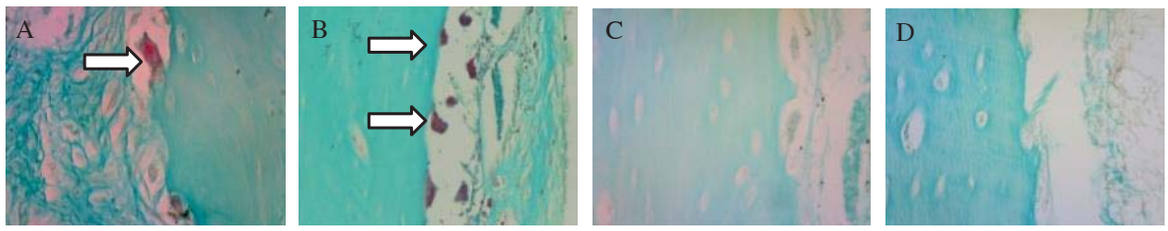

Figure 3. The results of histology test on periodontal tissues of the young guinea pigs, on the 7th day, at 400x magnification, with TRAP staining. A) the control group; B) the orthodontic treatment group; C) the genistein treatment group; D) the orthodontic + genistein treatment group.

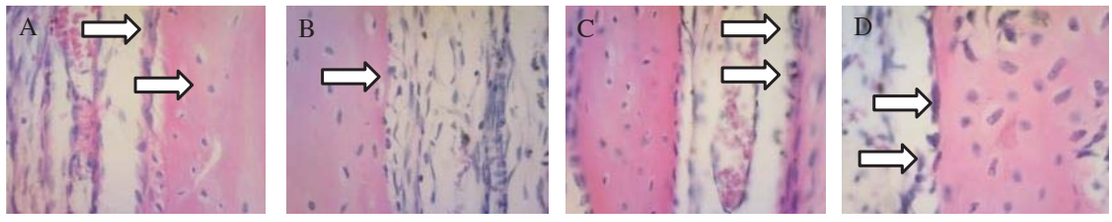

Figure 4. The results of histology test on periodontal tissues of the old guinea pigs, on the 7th day, at 400x magnification, with HE staining. A) the control group; B) the orthodontic treatment group; C) the genistein treatment group; D) the orthodontic + genistein treatment group.

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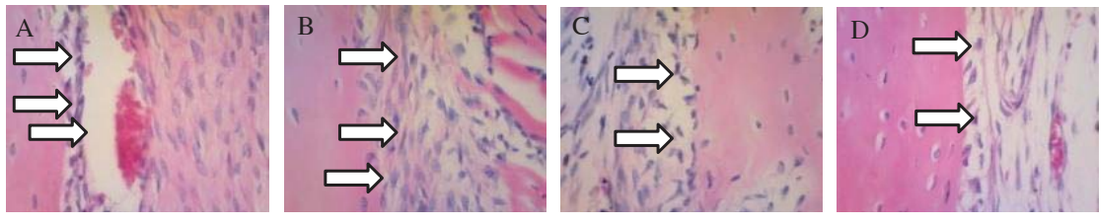

Figure 5. The results of histology test on periodontal tissues of the young guinea pigs, on the 7th day, at 400x magnification, with HE staining. A) the control group; B) the orthodontic treatment group; C) the genistein treatment group; D) the orthodontic + genistein treatment group.

Table 1. Means and standard deviations of osteoclasts on the pressure sites based on treatment and age

Age	Treatment			
	Control	Orthodontic	Genistein	Orthodontic + Genistein
Old	1.00± 1.26	10.67± 8.66	0	4.00± 6.20
Young	12.00± 9.59	20.17± 16.46	1.50± 2.34	0

Table 2. Results of the Mann-Whitney U test on the number of osteoclasts in the pressure sites based on treatment and age

Treatment		Control		Orthodontic		Genistein		Ortho+Genistein	
		Old	Young	Old	Young	Old	Young	Old	Young
Control	Old		1.00	0.872	0.028	0.059	1.00	1.00	0.059
	Young			0.872	0.470	0.007	0.037	0.096	0.007
Orthodontic	Old				0.297	0.007	0.02	0.242	0.007*
	Young					0.007	0.02	0.046*	0.007*
Genistein	Old						0.14	0.138	1.00
	Young							0.702	0.14
Ortho+ Genistein	Old								0.138
	Young								

Note: * There was a significant difference (p<0.05)

Table 3. Means and standard deviations of osteoblasts on the tension sites based on treatment and age

Age	Treatment			
	Control	Orthodontic	Genistein	Orthodontic + Gensitein
	Tension	Tension	Tension	Tension
Old	43.33± 19.46	55.33± 23.58	42.17± 33.24	104.17± 57.87
Young	78.83± 36.60	69.83± 39.10	135.17± 87.25	145.67± 44.88

The results of Kruskal Wallis test on all four treatments (the control group, the orthodontic treatment group, the genistein treatment group, and the orthodontic + genistein treatment group) showed that there was no significant difference in the number of osteoclasts with a p value of 0.0000. Based on the results, the highest number of osteoclasts in the surrounding of the pressure sites of those old guinea pigs was in the orthodontic treatment, followed with the orthodontic + genistein treatment group, the control group (osteoclasts seemed a bit), and the genistein treatment group (osteoclasts did not appear). It means that the lowest number of osteoclasts in the surrounding of the pressure sites of those old guinea pigs was in the genistein treatment group. On the other hand, the highest number of osteoclasts in the surrounding of the pressure sites of those young guinea pigs was in the orthodontic treatment, followed with the control group, the genistein treatment group, and the orthodontic + genistein treatment group (osteoclasts did not appear). It can be said that the lowest number of osteoclasts in the pressure sites of those young guinea pigs was in the orthodontic + genistein treatment group.

Moreover, the results of Mann-Whitney U test showed that there was a significant difference in the number of osteoclasts in the surrounding of the pressure site (p = 0.007)

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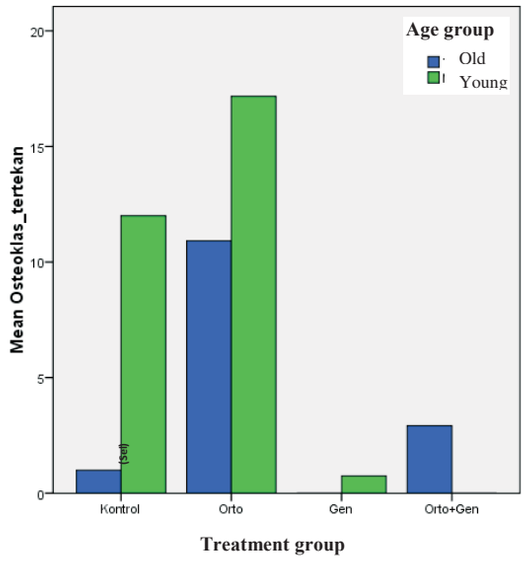

Figure 6. Mean number of osteoclasts on the pressure sites of young and old guinea pigs.

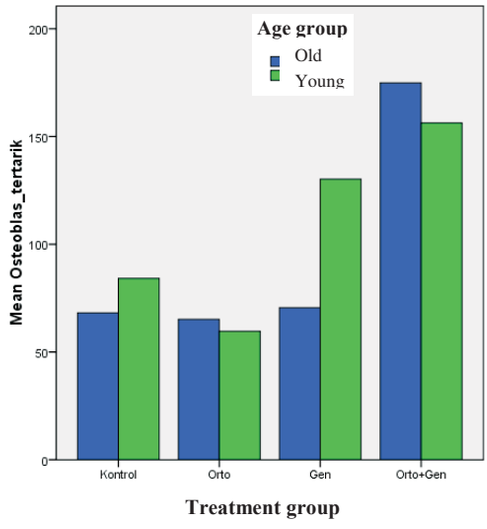

Figure 7. Mean number of osteoblasts on the tension sites of young and old guinea pigs.

between the orthodontic + genistein treatment group of the old guinea pigs and the orthodontic + genistein treatment group of the young guinea pigs. There was also a significant difference in the number of osteoclasts in the surrounding of the pressure site between the orthodontic treatment group of the young guinea pigs and the orthodontic + genistein treatment group of the young guinea pigs ($p = 0.007$). The number of osteoclasts in the orthodontic + genistein treatment group of the young guinea pigs was higher than the orthodontic treatment group of the young guinea pigs. Similarly, there was also a significant difference in the number of osteoclasts in the surrounding of the pressure site between the orthodontic treatment group of the young guinea pigs and the orthodontic + genistein treatment group of the old guinea pigs ($p = 0.046$) (Table 2).

Based on the results of the Kruskal Wallis test, furthermore, it is also known that there was a significant difference on the number of osteoblasts in the surrounding of the tension sites between all four groups (p=0.0000). Based on the results, the highest number of osteoblasts on the tension sites of those old guinea pigs was in the orthodontic+genistein treatment group, followed with the genistein treatment group, the control group, and the orthodontic treatment group. This means that the lowest number of osteoblasts in the surrounding of the tension sites of those old guinea pigs was in the orthodontic treatment group. On the other hand, the highest number of osteoblasts in the surrounding of the tension sites of those young guinea pigs was in the orthodontic+genistein treatment group, followed with the control group, the genistein treatment, and the orthodontic treatment group. This indicates that the lowest number of osteoblasts in the surrounding of the tension sites of those young guinea pigs was in the orthodontic treatment group.

In addition, the results of the Mann-Whitney U test showed that there was a significant difference ($p = 0.004$) in the number of osteoblasts in the surrounding of the tension sites between the orthodontic treatment group of the old guinea pigs and the orthodontic+genistein treatment group of the old guinea pigs. The number of osteoblasts in the

Table 4. Results of the Mann-Whitney U test on the number of osteoblasts in the tension sites based on treatment and age

Treatment		Control		Orthodontic		Genistein		Ortho+Genistein	
		Old	Young	Old	Young	Old	Young	Old	Young
Control	Old		0.423	0.419	0.688	0.297	0.037	0.01	0.02
	Young			1.00	0.336	0.149	0.261	0.006	0.037
Orthodontic	Old				0.376	0.335	0.054	0.004*	0.054
	Young					0.631	0.078	0.016*	0.025*
Genistein	Old						0.016	0.004	0.016
	Young							0.748	0.81
	Old								0.748
	Young								

Note: *There was a significant difference (p<0.05)

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orthodontic+genistein treatment group of the old guinea pigs was higher than in the orthodontic treatment group of the old guinea pigs. There was also a significant difference in the number of osteoblasts in the surrounding of the tension sites between the orthodontic treatment group of the young guinea pigs and the orthodontic+genistein treatment group of the old guinea pigs ($p = 0.016$). The number of osteoblasts in the orthodontic+genistein treatment group of the old guinea pigs was higher than in the orthodontic treatment group of the young guinea pigs. Similarly, there was a significant difference in the number of osteoblasts in the surrounding of the tension sites between the orthodontic treatment group of the young guinea pigs and the orthodontic+genistein treatment group of the young guinea pigs ($p = 0.025$). The number of osteoblasts in the orthodontic+genistein treatment group of the young guinea pigs was higher than in the orthodontic treatment group of the young guinea pigs (Table 4).

DISCUSSION

The results of Kruskal Wallis test showed that there were no significant differences in the number of osteoclasts ($p<0.05$) and in the number of osteoblasts ($p<0.05$) between the orthodontic treatment and the orthodontic+genistein treatment groups. Similarly, the results of the Mann-Whitney U showed that there was no significant difference (0.242) between the orthodontic treatment of the old guinea pigs and the orthodontic + genistein treatment of the old guinea pigs groups. This condition is probably caused by the fact that the observation was carried out on the 7th day. Osteoblasts and osteoclasts involved in bone formation will appear 40-48 hours after the provision of the orthodontic force.[13] Therefore, on the 7th day, the osteoclasts had been replaced with bone-forming cells, known as osteoblasts.

Based on the results, the highest number of osteoclasts in the surrounding of the pressure sites of those old guinea pigs was on the orthodontic treatment, followed with the orthodontic + genistein treatment, the control (osteoclasts seemed a bit), and the genistein treatment groups (osteoclasts did not appear). This condition is in accordance with a statement of Dang *et al*.[22] arguing that the estrogenic effects of isoflavone genistein involve a mechanism mediated by estrogen receptors. Isoflavone in this case acts as an estrogen stimulating osteogenesis, forming osteoblasts and inhibiting osteoclasts. As a result, in the orthodontic + genistein treatment group, the number of osteoclasts reduced, and even in the genistein treatment group, there was no any osteoclast. This is likely also due to the administration of genistein, considered as phytonutrients or phytoestrogens, have a chemical composition and function similar to estrogen. Some phytochemical compounds are also known as phytonutrients, including phytoestrogens considered as plant compounds that exist in daily diet. Phytoestrogens can positively regulate a number of physiological functions in mammalian systems, involved in chronic diseases such as osteoporosis.

On the other hand, the highest number of osteoclasts in the surrounding of the pressure sites of those young guinea pigs was in the orthodontic treatment, followed with the control group, the genistein treatment group, and the orthodontic + genistein treatment group. Osteoclasts even did not appear in the orthodontic + genistein treatment group of the young guinea pigs. Meanwhile, osteoclasts did not appear on the genistein treatment group of the old guinea pigs. This is in accordance with a statement of Dang and Lowik[23] arguing that the anti-estrogenic effect of isoflavones on micro molar concentrations cannot be explained only through its role when mediated by estrogen receptors, but also can be related to the results of the competition between estrogen and isoflavones to bind to the estrogen receptors.

In addition, the results of the statistical analysis on osteoblasts showed that there were significant differences ($p<0.05$) between the orthodontic treatment group of the old guinea pigs and the orthodontic + genistein treatment group of the old guinea pigs, between the orthodontic treatment group of the young guinea pigs and the orthodontic + genistein treatment group of the old guinea pigs, and also between the orthodontic treatment group of the young guinea pigs and the orthodontic + genistein treatment group of the young guinea pigs. On the other hand, there were not statistically significant differences in the number of osteoblasts between the orthodontic treatment group of the old guinea pigs and the orthodontic + genistein treatment group of the young guinea pigs. In overall, the highest number of osteoblasts was on the orthodontic + genistein treatment group. It is in line with a research conducted by Tsuda and Ushiroyama showing that *isflavones* have a variety of effects, such as increasing osteoblast activity and inhibiting osteoclast activity.[24,25] The effects of isoflavones on the number of osteoblasts in vitro actually have already been known.[12,19,22,27] Genistein is able to bind to β estrogen receptors in osteoblasts. DNA contained in osteoblasts, as a result, will increase significantly due to the addition of genistein or daidzein, triggering isoflavones to stimulate the proliferation of osteoblasts. Isoflavones can also increase the activity of alkaline phosphatase enzyme as a marker in the differentiation of osteoblasts.[26,27]

It can be concluded that the administration of soy isoflavone genistein can increase the number of osteoblasts in the surrounding of tension sites. The administration of soy isoflavone genistein also can decrease the number of osteoclasts, or can make osteoclasts not appear at all in the surrounding of pressure sites in both genistein treatment group of old guinea pigs and orthodontics + genistein group of young guinea pigs.

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